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TITLE: Transcriptomic Profiling and Functional Characterization of Fusion Genes in Recurrent Ovarian Cancer

PRINCIPAL INVESTIGATOR: Adrian V. Lee, Ph.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
                                       Pittsburgh, PA 15213

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Transcriptomic Profiling and Functional Characterization of Fusion Genes in Recurrent Ovarian Cancer

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W81XWH-16-1-0403

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pittsburgh
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

High-grade serous ovarian cancer (HGSOC) is known for its lack of early detection, limited therapies, and high rate of recurrence. Recent advances in transcriptomic sequencing have identified drug-targetable, pathogenic fusion genes in solid cancers. We hypothesize that fusion genes are commonly acquired or enriched in relapsed HGSOC and contribute to the enhanced malignancy observed in recurrent disease. In the first year of this proposal we have assembled a cohort of 18 patient matched pairs of chemotherapy naïve and resistant HGSOC and performed RNA sequencing. Every case showed acquisition of RNA fusions (average of 7) in the recurrent disease. Some fusions have biological functions in drug resistance, and some are found in HGSOC cell lines. We noted transcriptional similarity between the patient-matched pairs of samples, but several recurrent transcriptional remodeling events were noted. For the next year we will validate the clinical significance and biologic function of prioritized RNA fusion events.

15. SUBJECT TERMS

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1) INTRODUCTION:

High-grade serous ovarian cancer (HGSOC) is known for its lack of early detection, limited therapies, and high rate of recurrence. Greater than 80% of patients with late-stage HGSOC recur after an initial response to chemotherapy, with the majority of relapsed tumors developing deadly resistance to subsequent chemotherapies. The generation of fusion mRNA transcripts is an oncogenic event in many cancer types. Recent advances in transcriptomic sequencing have identified drug-targetable, pathogenic fusion genes in solid cancers. We hypothesize that fusion genes are commonly acquired or enriched in relapsed HGSOC and contribute to the enhanced malignancy observed in recurrent disease. The goal of this proposal is to test this hypothesis with the following specific aims; 1) To define the presence and relative expression of fusion mRNA transcripts in primary and recurrent high grade serous ovarian cancer (HGSOC). 2) To establish the prevalence and clinical importance of identified pathogenic gene fusions 3) To determine the biological effect, and mechanistic action, of fusion candidates acquired in relapsed disease. This study will provide novel targets and biomarkers for a cancer with limited options. This pilot project will develop key preliminary data critical for further analysis of RNA fusions in recurrent HGSOC and may identify new prognostic markers and ultimately therapeutic targets for reversing HGSOC chemoresistance, reducing recurrence, and extending patient survival.

2) KEYWORDS:

High grade serous ovarian cancer, chemotherapy resistance, RNA fusions, prognosis, recurrence, sequencing

3) ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1) Identify fusion transcripts in recurrent HGSOC	Timeline	Progress
Major Task 1 RNA-sequencing of recurrent HGSOC	Months	
Local IRB/IACUC Approval	0	Completed
Submission of institution's IRB approval and related material for DoD's HRPO approval	0-1	Completed
Receive HRPO approval or exempt finding before initiating relevant tasks	1-3	Completed
Subtask 1 Pathology analysis of 20 pairs of primary and recurrent HGSOC	3-4	Completed
Subtask 2 Isolation of RNA from 20 pairs	3-5	Completed

Subtask 3 Sequencing using NextSeq500 in sequencing core at Pitt	5-6	Completed
Milestone(s) Achieved – RNA-seq data from primary and recurrent Pitt tumors (n=23 total – 3 pairs performed for preliminary data)		Completed
Major Task 2 Bioinformatic analysis and validation of fusions		
Subtask 1 Analysis of RNA-seq by mapping with STAR and calling fusions using Fusion MetaCaller. Analysis of gene expression using STAR and Deseq	6-7	Completed
Subtask 2 Validation of candidate fusions using RT-PCR and Q-RT-PCR	7-8	Partial – in progress
Subtask 3 Validation of a select number of fusions by FISH	8-10	Not started
Milestone(s) Achieved: Validated fusion mRNAs present in recurrent HGSOC		Partial – in progress
Specific Aim 2) Establish the prevalence and clinical significance of identified fusion genes		
Major Task 3 Isolate RNA and measure fusion using Nanostring		
Subtask 1 Procure 60 FFPE recurrent samples and 200 primary HGSOC	4-8	Partial – in progress
Subtask 2 Isolate and measure RNA from 260 samples	8-10	Partial – in progress
Subtask 3 Develop NanoString codeset based upon fusions from Aim 1	10-14	Not started

What was accomplished under these goals?

For this reporting period describe:

1) major activities

The major activity of the first year of funding has been to obtain frozen samples of patient-matched pairs of chemotherapy naïve and resistant (recurrent) high grade serous ovarian cancer and then perform RNA sequencing to identify RNA fusions. We believe that the cohort

we have assembled is the largest cohort of matched pair to date, and thus likely to reveal entirely new information on chemotherapy resistance HGSOC. We have performed the sequencing and identified RNA fusions, some of which are present in multiple tumors and also in ovarian cancer cell lines. We have performed initial validation of some of these fusions and this work is ongoing. Further details of the results are provided in part 3 below.

2) specific objectives

The major tasks of the first year were 1) RNA-sequencing of recurrent HGSOC, 2) Bioinformatic analysis and validation of fusions, and 3) 3 Isolate RNA and measure fusion using Nanostring

3) significant results or key outcomes, including major findings, developments, or conclusions

a) Local IRB/IACUC Approval

Written and approved

b) Submission of institution's IRB approval and related material for DoD's HRPO approval

Written and approved

c) Receive HRPO approval or exempt finding before initiating relevant tasks

Written and approved

d) Subtask 1 Pathology analysis of 20 pairs of primary and recurrent HGSOC

We identified cases of HGSOC suitable for this study. Inclusion criteria for this study were (1) patients harbored patient-matched frozen tissue from primary ovarian cancer and a later recurrence (referred to as "early" and "late" disease respectively (2) biospecimens contained regions with sufficient tumor cellularity (> 30%, median in cohort 80%) by pathologic analysis. Both a top and bottom slide of the whole tumor, with RNA extraction slides in between, were reviewed by a trained molecular pathologist to confirm pathology and to quantify tumor cellularity. 19 cases were identified (Table 1) but one was removed for quality control issues thus leaving a total of 18 cases.

Table 1 shows the clinical characteristics of the cohort.

Case	Disease Interval (months)	Early Disease Site	Late Disease Site
OVCA_01	32	Ovary	Small Bowel
OVCA_02	22	Omentum	Met NOS
OVCA_03	72	Met NOS	Colon
OVCA_04	24	NA	Lymph Node

OVCA_05	37	NA	Met NOS
OVCA_06	48	Ovary	Ovary
OVCA_07	88	Met NOS	Abdominal Wall
OVCA_08	18	Omentum	Lymph Node
OVCA_09	73	Spleen	Ovary
OVCA_10	62	Ovary	Met NOS
OVCA_11	24	NA	NA
OVCA_12	37	Ovary	Lymph Node
OVCA_13	25	Ovary	Colon
OVCA_14	55	Ovary	Abdominal Wall
OVCA_15	54	Ovary	Lymph Node
OVCA_16	6	Omentum	Spleen
OVCA_17	7	Omentum	Spleen
OVCA_18	6	Endometrium	Abdominal Wall
OVCA_19	64	Ovary	Pelvic Mass

One case was eliminated for quality control reasons leaving 18 cases (OVCA 1-19).

e) Subtask 2 Isolation of RNA from 20 pairs

Six, 25-micron frozen OCT-embedded sections were pooled and underwent RNA extraction using Qiagen's RNeasy protocol. Nucleic acids were quantified fluorometrically with a Qubit 2.0 Fluorometer and quality assessed with an Agilent 4200 TapeStation Instrument to determine RIN scores prior to sequencing. All samples had a RIN score above 7.5 and this made them suitable for downstream sequencing.

f) Subtask 3 Sequencing using NextSeq500 in sequencing core at Pitt

RNA-seq library preparation was performed for 18 early and late disease ovarian cancer pairs using approximately 500 ng of RNA and Illumina's TruSeq Stranded Total RNA-seq with Ribo-depletion protocol. Indexed, pooled libraries were then sequenced on High Output flow cells using an Illumina NextSeq 500 system (paired-end reads, 2 X 150 bp). A target of 50 million reads per sample was used to plan indexing and sequencing runs. Quality control metrics were excellent.

Major Task 2 Bioinformatic analysis and validation of fusions

g) Subtask 1 Analysis of RNA-seq by mapping with STAR and calling fusions using Fusion MetaCaller

Algorithms for identifying RNA fusions are changing rapidly. Based upon our data and others we chose to use an algorithm FusionCatcher which has very high specificity. Fusion RNAs were called with FusionCatcher v0.99.7b. Default parameters were used. Final-candidate fusion genes were subsequently filtered for cancer-specific fusions by discarding any fusion also detected in the Human Protein Atlas350 or BodyMap (EMBL-BMI, E-MTAB-513) RNA-sequencing datasets. The same fusion analysis was performed on ovarian cancer cell line data from the public Cancer Cell Line Encyclopedia (CCLE).

We identified fusions in the chemotherapy naïve (early, E) and recurrent (late, L) HGSOC. After excluding fusion RNAs found in a comprehensive panel of normals, a median of 7 cancer-specific fusion transcripts was acquired in each late disease sample. Nearly all recurrences also harbored "preserved" fusions—fusion transcripts detected in both the early and late lesion (Figure 1A). 152 fusions were predicted to produce an in-frame, chimeric protein—48 being acquired in late disease and 55 being preserved.
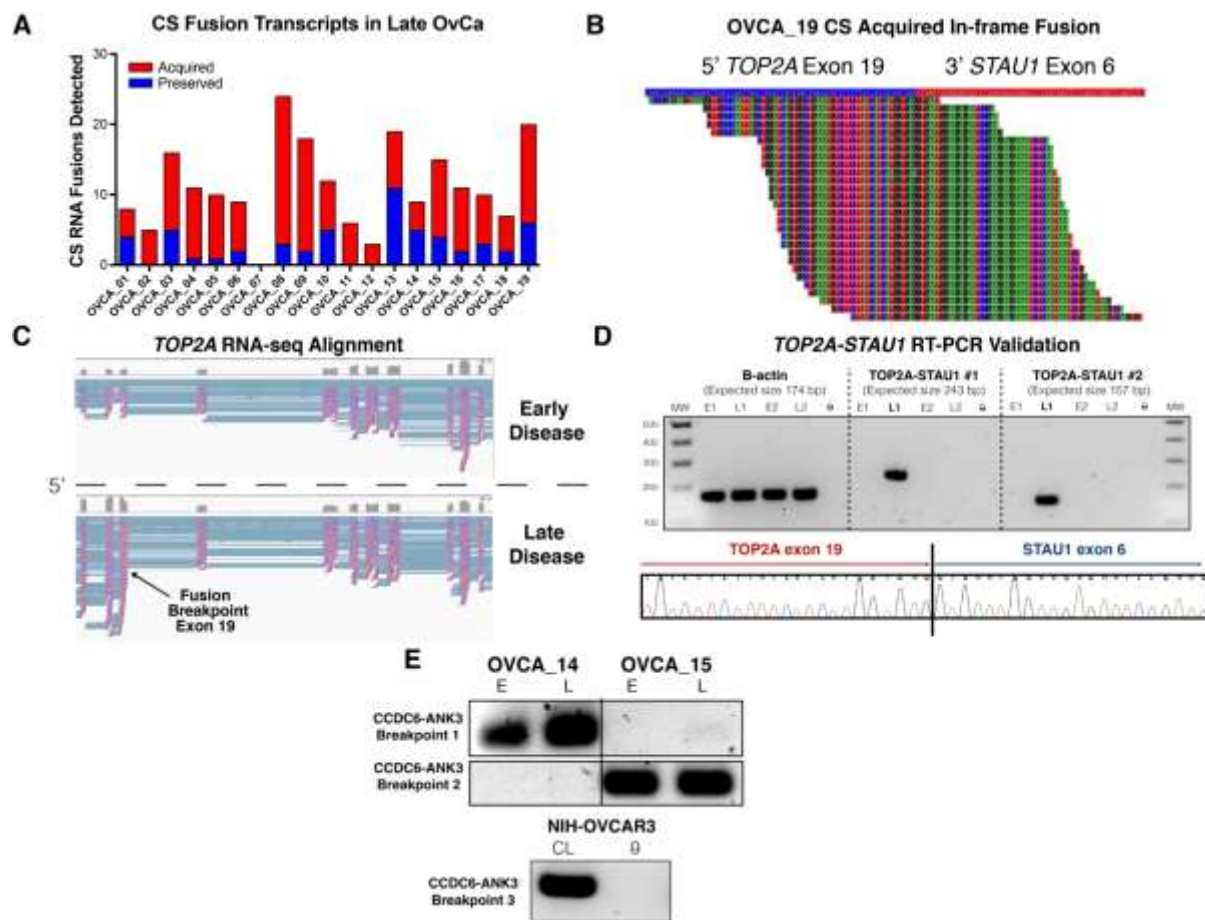

Figure 1: Fusion RNA landscape in recurrent ovarian cancer

(A) Landscape of cancer-specific (CS) fusion transcripts in late ovarian cancer. Frequency of cancer-specific fusions are shown for each case, with blue representing the number of preserved fusions (present in both early and late

disease) and red representing the number of late disease acquired fusions. (B) Reads supporting the in-frame, late disease specific fusion involving TOP2A and STAU1. (C) STAR RNA-seq alignment showing enriched coverage of reads preceding TOP2A fusion breakpoint at exon 19. (D) RT-PCR of ACTB and TOP2A-STAU1 in early and late disease samples of case OVCA_19 (E1, L1) and another, unrelated early and late disease pair (E2, L2). Sanger sequencing of PCR product showing fusion breakpoint sequence below gel image. (E) CCDC6-ANK3 fusion validations. Top: Ovarian cancer cases, E = early disease, L = late disease sample. Bottom: OVCAR-3, CL = cell line, 0 = no template control.

Although no acquired fusions were present in more than one recurrence, fusions of particular interest included an acquired *WNT2-CTTNBP2* in case OVCA_04, which retained a Wnt signaling peptide in the N-terminal region of the hypothetical protein product, and a fusion involving *TOP2A* (chromosome 17) and *STAU1* (chromosome 20) in case OVCA_19. Given the latter fusion's involvement with a known chemoresistance mediator, *TOP2A,* we explored this fusion in more detail. The *TOP2A-STAU1* fusion, containing up to exon 19 in *TOP2A* and the 3' region of *STAU1* beginning at exon 6, carried a high degree of bioinformatic support with 19 unique reads spanning the breakpoint (Figure 1B). Visualization of the RNA-seq alignment also revealed increased coverage of *TOP2A* up until the breakpoint in only the late disease sample (Figure 1C). The fusion was then validated with RT-PCR and Sanger sequencing using two separate PCR primer pairs spanning the breakpoint. Importantly, *TOP2A-STAU1* was not detected in the early lesion or in an unrelated sample—confirming its specificity to OVCA_19 and its acquisition in advanced disease (Figure 1D).

Because preserved fusions we found to be common in ovarian cancer recurrences, we searched for preserved fusion genes that were shared in multiple samples, which would increase their likelihood of being driver alterations. Two recurrent in-frame fusions were identified—MED12-IRF2BPL and CCDC6-ANK3. The bioinformatically called MED12-IRF2BPL fusion breakpoint was within highly homologous polyglutamine repeat regions of each fusion partner, suggesting this as a false positive fusion. CCDC6-ANK3; however, was found to harbor distinct breakpoints in each of the samples called—all of which produced a hypothetical, in-frame protein product. These breakpoints were confirmed with RT-PCR and another CCDC6-ANK3 fusion was validated in the cisplatin-resistant OVCAR3 cell line (Figure 1E)

h) Analysis of gene expression using STAR and Deseq

To determine global transcriptome differences between matched pairs, unsupervised hierarchical clustering was performed using normalized expression values. Nine pairs clustered in the same doublet clade of their patient-matched primary, suggesting a profound transcriptional conservation between the recurrence and the early lesion (Figure 2A). To confirm samples were patient-matched, given up to 88 months between early and late disease surgeries, an analysis of shared variants was performed. All pairs harbored a higher proportion of shared variants with their patient-matched primary than to other samples (Figure 2B).
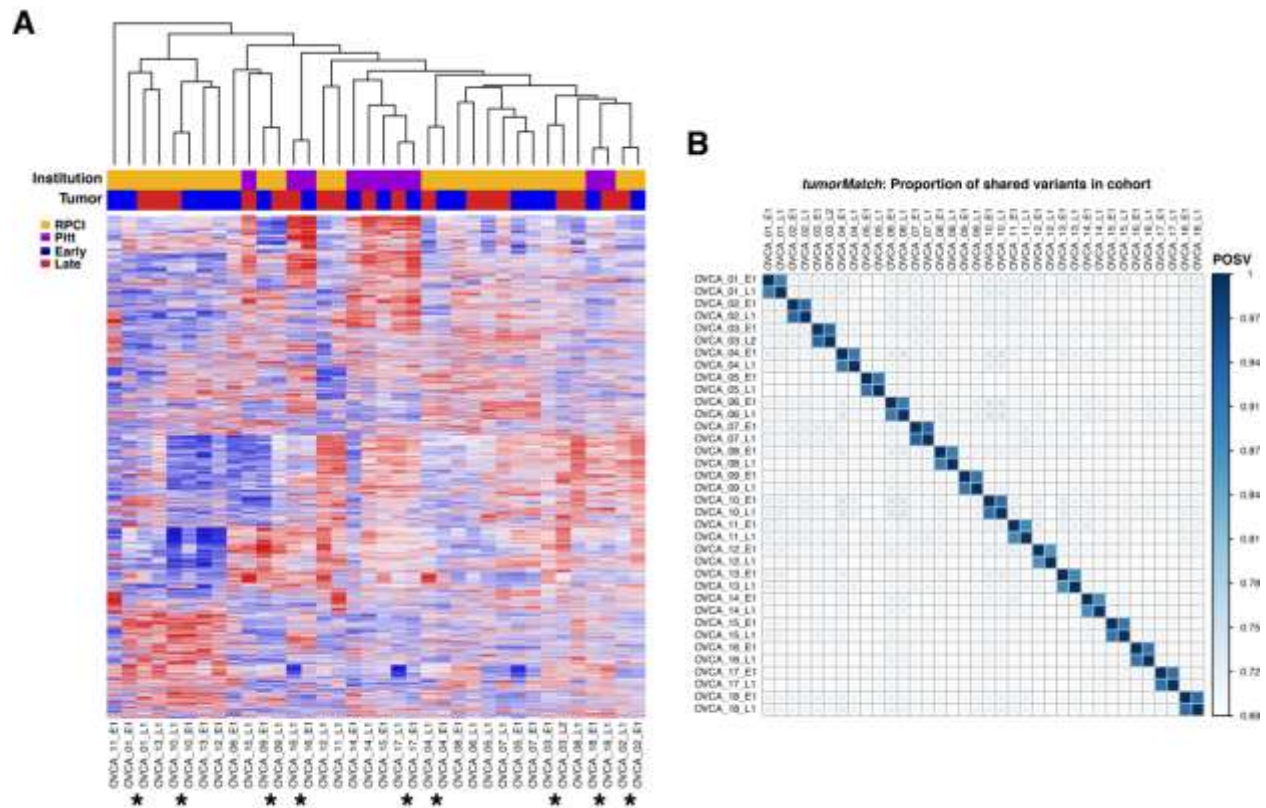
Figure 2: Unsupervised clustering and tumorMatch in ovarian cancer cohort

(A) Unsupervised hierarchical clustering on 20% most variable genes across the cohort (E1 = early disease, L1/2 = late disease). Institution (yellow = Roswell Park Cancer Institute; purple = University of Pittsburgh) and tumor type (blue = early disease; red = late disease) is indicated. Samples marked with an asterisk are early and late lesions that cluster together. (B) tumorMatch scores which represent the proportion of shared variants between samples. Darker blue and larger squares indicate a higher degree of genetic similarity between samples.

Differential expression analyses revealed heterogeneous expression between the patient-matched samples, only uncovering 39 differentially expressed genes (Figure 3A). The most significantly upregulated gene in late ovarian cancer was NTRK2, showing upregulation in the majority of recurrences (Figure 3B). Other genes included a suite of adipogenesis genes, such as FABP4, ADIPOQ, APOD, and upregulation of an ABC transporter, ABCA6.
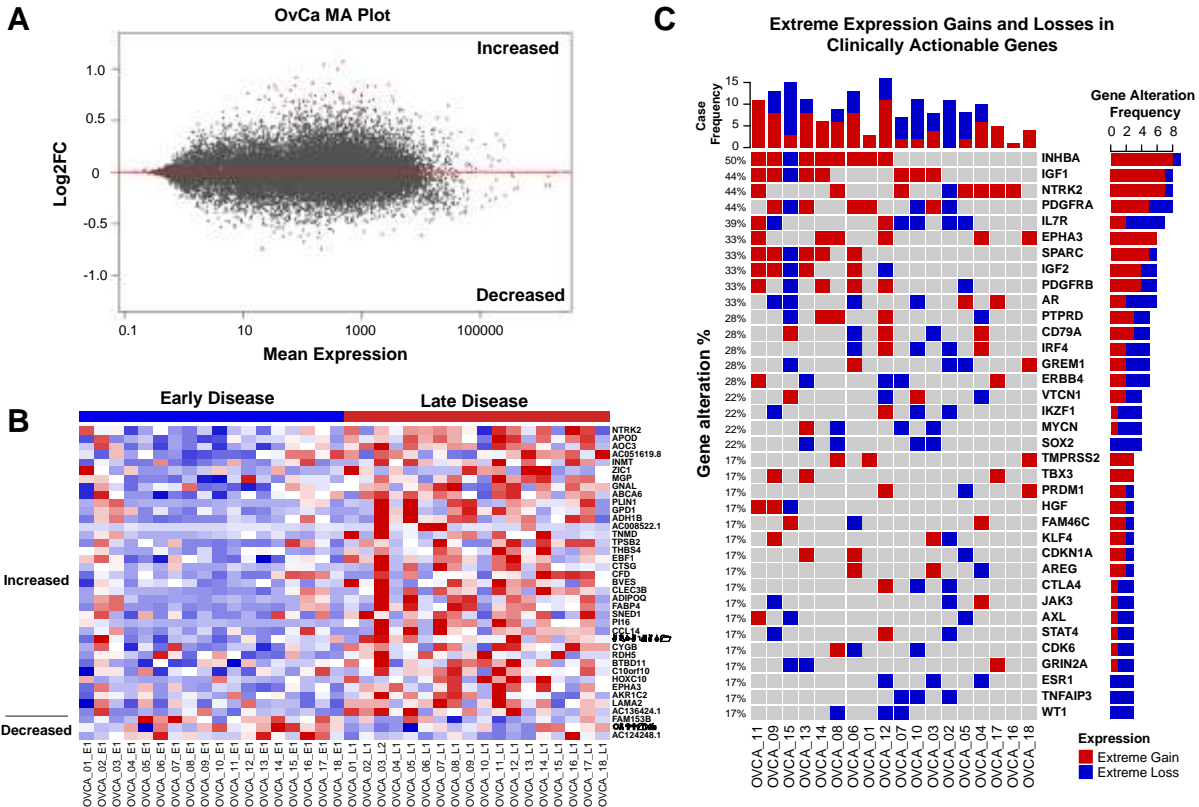
Figure 3: Differentially expressed genes and outlier expression events in ovarian cancer recurrences

(A) MA plot of genes interrogated for differential expression (log2FC [recurrence vs. primary] vs. mean of normalized counts). Significant genes (n = 39, padj < 0.10) are indicated in red. (B) Normalized expression heatmap of differentially expressed genes, ranked by adjusted p-value (top to bottom) and segregated by increased genes and decreased genes. (C) OncoPrint of outlier expression gains (red) and losses (blue) in patient-matched pairs, along with alteration case frequencies and recurrence percentages.

Since resistance mechanisms in advanced cancers may be mutually exclusive, and thus would be missed by conventional differential expression analyses given the gene-level stringency, we performed a targeted analysis focusing on outlier expression gains and losses—particularly in genes that are clinically actionable. Four clinically actionable genes showed outlier increases in at least one-third of late disease samples versus their matched early disease lesion—INHBA, IGF1 NTRK2 and EPHA3 (Figure 3C).

i) Subtask 2: Validation of candidate fusions using RT-PCR and Q-RT-PCR

In addition to the fusions that were validated above using RT-PCR, we selected another three, bioinformatically identified "acquired" fusion RNAs to validate using RT-PCR with primers flanking the breakpoints. All three were found to be either specific to the recurrence or highly enriched in the recurrence versus the matched primary (Figure 4). This gives us confidence in both the RNA sequencing and the fusion calling algorithm,
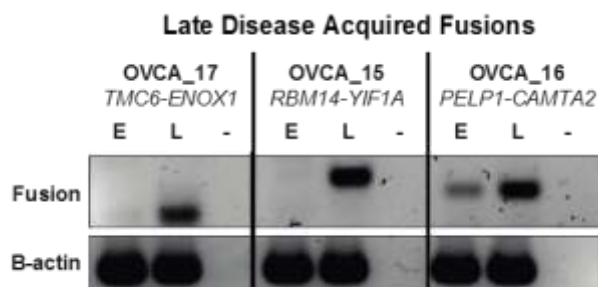
Figure 4: RT-PCR validation of late-disease 'acquired' fusions.
Three bioinformatically called fusion RNAs validated with RT-PCR using fusion breakpoint flanking primer pairs.
Case and fusion are indicated, E = early disease sample, L = late disease sample.

J: Subtask 3 Validation of a select number of fusions by FISH

This has not yet been started due to the technical challenges of FISH and our desire to test fusions which have clinical importance. We will perform this aim on fusions found to affect prognosis from Specific Aim 2.

Specific Aim 2) Establish the prevalence and clinical significance of identified fusion genes

Major Task 3 Isolate RNA and measure fusion using Nanostring

k) Subtask 1 Procure 60 FFPE recurrent samples and 200 primary HGSOC

This is in progress. The identification of the HGSOC for use in this project has proven challenging and taken more time than expected. We have identified 1,006 cases of HGSOC treated at our hospital, and have extracted clinical information on all of them. Importantly, we identified 174 cases which received neoadjuvant chemotherapy and thus would allow us to examine changes in fusion expression before and after neo-adjuvant chemotherapy. We are currently determining how many of these had a biopsy before and after surgery at our hospital and will request these tissues. Furthermore, for the other cases (n=832) we are currently identifying which ones had primary debulking, adjuvant chemotherapy and then had biopsy proven recurrent disease. We will then request these tissue for analysis. We expect to make this request in the next month.

l) Subtask 2 Isolate and measure RNA from 260 samples

This has not yet been started as we don't have the samples.

m) Subtask 3 Develop NanoString codeset based upon fusions from Aim 14) other achievements. Include a discussion of stated goals not met.

This has not yet been started as we are finalizing the prioritized list of fusions to analyze. One challenge is the process of prioritizing fusions for further study. Since submitting the original proposal, several machine learning algorithms have been developed which can predict the biological significance of bioinformatically predicted fusions (such as OncoFuse, Pegasus, Fusion

Centrality). These are described at this website; https://omictools.com/driver-gene-fusion-prediction-category. We have implemented OncoFuse and Pegasus and initial results with Oncofuse show excellent prediction of known fusions. We will implement this for this identified fusions to predict which ones are likely important and should be studies further.

n) What opportunities for training and professional development has the project provided?

Nothing to report

o) How were the results disseminated to communities of interest?

Nothing to report

p) Describe how the results were disseminated to communities of interest.

Nothing to report, however while not in this reporting period, we will present the work as a poster at the upcoming AACR Addressing Critical Questions in Ovarian Cancer Research and Treatment, October 1 - 4, 2017, Wyndham Grand Pittsburgh.

q) What do you plan to do during the next reporting period to accomplish the goals?

We will obtain the next batch of clinical samples and continue study of the fusions and thus complete the goals and tasks for specific aim 2.

r) Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Specific Aim 2) Establish the prevalence and clinical significance of identified fusion genes

Major Task 3 Isolate RNA and measure fusion using Nanostring

Major Task 4 Bioinformatic analysis of NanoString data

Major Task 5 Development of HGSOC cell lines with knockdown or overexpression of fusion genes

Major Task 6 Examine the phenotype of HGSOC cell lines with and without gene fusions

4) IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We are the first to identify RNA fusions in chemotherapy naïve and resistant HGSOC, and the finding of acquired fusions in all of the cases suggests there maybe biological drivers of recurrence. This is a novel and new finding. The next period of work will be key to decipher which of these RNA fusions is key to HGSOC. The gene expression findings are also novel and may highlight several new therapeutic targets which once reported will all be publicly available and can be studied by others.

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project.

What was the impact on other disciplines?

Nothing to report

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

We are using new methods for RNA fusion discovery, validation, and then driver prediction which will add to others work and help guide this field forward. For example, the use of RNA fusion driver prediction algorithms is nascent, and our prediction and then functional validation will deliver data and help improve these algorithms and approaches.

What was the impact on technology transfer?

Nothing to Report

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

Nothing to Report

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

Nothing to report

5) CHANGES/PROBLEMS:

Describe any changes in approach during the reporting period and reasons for these changes

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

We had an actual delay in the procurement of the second set of human HGSOC samples. The identification and procurement of human samples is always a challenge. Samples must be identified and then clinical characteristics identified to make sure that the correct samples are procured. However, we now have the patient cohort identified and will now request the tissues. There is a possibility that some cases will not have available tissue, however, we have such a large number of cases identified (>1000) that such a loss shouldn't present a problem.

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Delays in identifying and procuring tissue as noted above. We now have the patients identified and do not expect further issues.

Changes that had a significant impact on expenditures

Nothing to report

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period.

Nothing to report

6) PRODUCTS:

Nothing to Report

Publications, conference papers, and presentations

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7) PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Adrian V. Lee, Ph.D.

Project Role: Principle Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr. Lee has overseen the whole project and directed the research.

Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Name: Peter Lucas

Project Role: co-Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr Lucas is a breast and ovarian cancer molecular pathologist. Dr Lucas reviewed all cases, and assessed cellularity and suitability of samples for further analysis. He also participated in data interpretation.

Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Name: Robert Edwards

Project Role: co-Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr. Edwards has provided advice on HGSOC and clinical implications of the data. He has met with Dr Lee and his team regularly to advise on the progress and future direction. He gives invaluable advice on the clinical relevance of the studies and interpretation of the data.

Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Name: George Tseng

Project Role: co-Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr. Tseng is a biostatistician who provides advice and support on the bioinformatics aspects of the project. Together with Dr Lee and Priedigkeit they have published on RNA fusion algorithms. Dr Tseng provides advice on data analysis and supervises Li Zhu a graduate student on the project.

Name: Nick Smith

Project Role: graduate student

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 3

Contribution to Project: Nick Smith is a graduate student who has helped with bioinformatic analysis of data.

Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Name: Li Zhu

Project Role: graduate student

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 3

Contribution to Project: Li Zhu is a biostatistics graduate student who worked on the project. She has helped Nolan Priedigkeit with concepts and analysis of biomedical data.

Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Name: Nolan Priedigkeit

Project Role: graduate student

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 12

Contribution to Project: Nolan Priedigkeit is a MSTP student who worked on the project. He isolated RNA, performed sequencing, and analysis of the data

Funding Support: Nolan is supported by a NIH grant F31CA203095. No funds from this grant were used to support his effort

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization Name: Roswell Park Cancer Institute (RPCI)

Location of Organization: Buffalo, NY

Partner's contribution to the project (identify one or more)

Financial support; N/A

In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff); N/A

Facilities (e.g., project staff use the partner's facilities for project activities); N/A

Collaboration (e.g., partner's staff work with project staff on the project); RPCI will analyze the RNAseq data in collaboration following transfer under MTA/DUA. This work is in-kind and no financial support is provided.

Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); N/A

8) SPECIAL REPORTING REQUIREMENTS

Nothing to report

9) APPENDICES

Nothing to report